## 803 Angioplasty and Restenosis: Basic II

Wednesday, March 27, 1996, 4:00 p.m.–5:00 p.m.
Orange County Convention Center, Room 224F

4:00

### 803-1 Neointimal Formation Is Dependent on the Underlying Arterial Substrate After Coronary Stent Placement

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Restenosis is more frequent after stent placement in patients with prior PTCA. The pattern and severity of neointimal hyperplasia after stent placement may depend on the underlying plaque morphology. We compared histology and smooth muscle cell proliferation at 2 months after stent placement in normal and atherosclerotic porcine coronary arteries to determine if the presence of an atherosclerotic plaque influenced neointimal formation. Atherosclerotic lesions were induced by high cholesterol diet and overstretch balloon injury to produce an eccentric fibrocellular plaque. Four weeks after balloon injury, 8 stainless steel balloon expandable stents (*MULTILINK*™) were implanted (stent to artery ratio 1.04 ± 0.05) in the coronary arteries of 8 swine. Six stents were implanted in a similar manner in the coronary arteries of 3 normolipemic swine. Histology demonstrated a significantly greater mean neointimal area in the atherosclerotic (3.24 ± 0.26 $mm^2$) than in the normal (1.84 ± 0.34 $mm^2$, p = 0.006) arteries. The mean neointimal thickness was also significantly greater in the atherosclerotic (0.38 ± 0.03 mm) versus the normal (0.19 ± 0.05 mm, p = 0.003) arteries. In the atherosclerotic arteries, the neointimal thickness was maximal at the stent wire sites in the region of the underlying fibrocellular plaque (0.48 ± 0.02 mm) and least where the internal elastic lamina was intact with normal media (0.27 ± 0.01 mm, p < 0.0001). The PCNA labeling index of smooth muscle cells was greatest within the plaque beneath the stent wires (18.2 ± 1.6) as compared to the neointima within the stent (3.4 ± 0.6 atherosclerotic, 2.6 ± 0.8 normal) or the media (1.7 ± 0.6 atherosclerotic, 0 normal) (p < 0.001).

Neointimal formation after stent placement is increased in the presence of a plaque that has a sustained and high level of smooth muscle cell proliferation. These data may be useful in understanding the mechanism of stent restenosis in patients with prior PTCA.

4:15

### 803-2 Prolonged Thrombin Inhibition Reduces Restenosis After Balloon Angioplasty in Porcine Coronary Arteries

Richard Gallo, Vincenzo Toschi, John T. Fallon, James H. Chesebro, Valentin Fuster, Juan J. Badimon. *Cardiovascular Biology Laboratories, Mount Sinai Medical School, New York, NY*

Thrombin is generated in large quantities after arterial injury. It is a powerful mitogen for smooth muscle cells *in vitro*, and a potent stimulus for platelet activation. Hirudin is highly effective in preventing acute platelet-rich thrombosis after deep arterial injury as compared to heparin. This study was designed to test whether prolonged specific inhibition of thrombin limits restenosis after balloon angioplasty (BA) in porcine coronary arteries. BA was performed in Yorkshire Albino pigs at a balloon to vessel ratio of 1.7:1. At BA animals were assigned to either heparin 100 units/kg, i.v. bolus, (n = 8) or Hirudin 0.7 mg/kg, i.v. bolus, followed by 0.7 mg/kg/hr for 14 days via continous i.v. infusion, *(Medtronic Synchromed pump)*, (n = 7). aPTT ratios were maintained 2–3 times above control levels in the hirudin group. Coronary arteries were perfusion fixed 28 days after BA. Histomorphometric measurements were performed on deeply injured sections by computerized analysis of luminal, medial, neointimal (including residual thrombus, hematoma, and fibromuscular hyperplasia), and total vessel areas. Blinded planimetric analysis showed decreased neointimal thickening in segments treated with hirudin compared to those treated with heparin alone (37.8% ± 5 vs 61.0% ± 4, p = 0.004) a 61.9% relative reduction in luminal narrowing. Hirudin treatment also reduced the contribution of organizing thrombus and fibromuscular hyperplasia to luminal narrowing from 13.3% ± 2 to 4.5% ± 1 (p = 0.01), and 42.2% ± 3 to 28.7% ± 3 (p = 0.02), respectively. Residual hematoma (thrombotic component of neointima under a medial flap or tear) was unaffected by hirudin. In conclusion prolonged specific inhibition of thrombin by r-hirudin significantly reduces neointimal thickening as measured by quantitative histopathology in porcine coronary arteries. This beneficial effect is mediated by an inhibition of both the fibromuscular and thrombotic response to arterial injury.

4:30

### 803-3 17-β Estradiol Inhibits Neointima Formation After Coronary Angioplasty in Swine

Ronald L. Levine, Peter G. Anderson, Kerry Lyle, Sanjay S. Yadav, Suzanne Oparil, Gary S. Roubin. *University of Alabama, Birmingham, Alabama*

This study tested the hypothesis that 17-β estradiol inhibits neointima formation after balloon injury in the coronary arteries of swine. Twenty-one pigs (11 ♂, 10 ♀) were randomized to treatment or control groups. Thirty day sustained release pellets containing either 1400 mg of 17-β estradiol or placebo were implanted subcutaneously. Six days later, animals underwent angioplasty of the circumflex artery. Five inflations to 10 atm for 30 sec were performed with compliant balloons. Pigs were sacrificed 2 weeks after balloon injury. Areas were calculated utilizing a morphometric analysis program. Arteries were assigned an injury score based on the degree of injury. Results (mean ± sem):

| | ♂ Estradiol n = 5 | ♂ Placebo n = 6 | ♀ Estradiol n = 5 | ♀ Placebo n = 5 |
|---|---|---|---|---|
| Intima area ($mm^2$) | 0.46 ± 0.14 | 0.95 ± 0.10** | 0.37 ± 0.11 | 1.01 ± 0.12*** |
| Intima thickness (μm) | 467 ± 52 | 614 ± 68** | 207 ± 136 | 448 ± 74* |
| Injury score (0–3) | 1.59 ± 0.24 | 1.64 ± 0.21 | 1.86 ± 0.09 | 1.96 ± 0.07 |
| Serum estradiol (pg/ml) | 1003 ± 130 | < 15**** | 605 ± 121 | < 15**** |

*p < 0.05, **p < 0.02, ***p < 0.005, ****p < 0.001 estradiol vs. placebo

*Conclusions:* 17-β estradiol reduces neointima formation after balloon injury of the coronary artery in ♂ and ♀ pigs. This intervention may be useful in the prevention of urestenosis in patients.

4:45

### 803-4 Initial Characterization of the Temporal Relationship Between Procollagen and TGF-β1 Gene Expression During Post-Angioplasty Arterial Repair

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Transforming growth factor (TGF-β1) is a recognized in vivo modulator of neo-intimal (INT) growth, influencing both smooth muscle cell (SMC) proliferation & extracellular matrix (ECM) protein synthesis. In order to define the time course and temporal relationship of changes in the genetic expression of procollagens & TGFβ1 during arterial repair, atherogenic rabbits underwent bilateral iliac artery endothelial stripping, followed 2 weeks later by angioplasty ([AP], 6 ATM. × 60 sec × 2). Animals were serially sacrificed at days 2 (n = 4), 7 (n = 4) and 30 (n = 3). Iliac artery AP zones were analyzed biochemically for total tissue proteins, DNA, RNA, Hydroxyproline (Hyp) and mRNA expression of procollagens α1 (I), α1 (III), & TGFβ1. Means of the relative densitometric mRNA expression were normalized to the constitutively expressed GAPDH mRNA. Contralateral (pressure-fixed) vessels were analyzed by quantitative histomorphometry. *Results:* Tissue total collagenous protein content increased significantly from day 2 to 30 (p = 0.01), with a concomittant reduction in luminal cross-sectional area (CSA, p = 0.002). However this was not associated with a significant change in total DNA and RNA contents of the vessel wall (P = NS). Procollagens I, III and TGFβ1 to GAPDH mRNA ratios rose significantly from day 2, to peak at day 30 (p = < 0.05). The rise in the genetic expression of TGFβ1 and procollagen α1 (I), during the repair phase was correlated with at 2, 7 and 30 days.

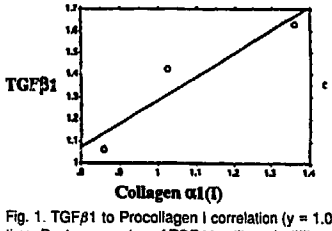


Fig. 1. TGFβ1 to Procollagen I correlation (y = 1.05873x + 0.22754, $r^2$ = 0.87513) with time. Peak expression of TGFβ1, α(I), and α(III) was observed at day 30.

*Conclusions:* These data demonstrate a tight temporal concordance between TGFβ1 and procollagen I & III gene expression which preceeds the increase in neointimal collagenous matrix content and vascular tissue growth following barotrauma injury. This relationship suggests genetic comodulation of vascular structural protein synthesis during tissue repair.